LIPID METABOLISM IN *BLATTELLA GERMANICA* L. : COMPOSITION DURING EMBRYONIC AND POST EMBRYONIC DEVELOPMENT

Y.S. KRISHNAN
Department of Entomology
University of Alberta
Edmonton, Canada

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Changes in the lipid composition of Blattella germanica *L. during embryonic and post embryonic development were investigated by a combination of column, thin-layer and gas-liquid chromatography. During embryogenesis the loss of dry matter was mainly due to utilization of triglycerides. Hydrocarbon and sterol content increased slightly. Mono- and di-glyceride and free fatty acid content increased substantially. Fatty acid analysis revealed the presence of 17 fatty acids ranging in chain length from C_6 to C_{22}. Oleic acid was the most abundant followed by palmitic acid. Phospholipid content increased during embryonic and post embryonic development. Lecithin, cephalin and sphingomyelin were the major phospholipids. During nymphal development all the lipid fractions increased approximately in proportion to the increase in body weight. Nymphs and adults had similar lipid composition.*

Lipids include neutral fats, phospholipids, cerebrosides, sterols and fat soluble vitamins. Many earlier works on insect lipid were restricted to gross quantitative analysis. The chemistry of insect fats has been reviewed by Timon-David (1930), Scoggin and Tauber (1950) and Hilditch (1956). In the past decade the volume of literature on the lipid composition of insects has increased tremendously as is evidenced by the publication of a number of reviews (Niemierko 1959, Babcock and Rutschky 1961, Gilmour 1961, Kilby 1963, Fast 1964, Gilby 1965, Kinsella 1966b, Gilbert 1967a). The remarkable growth in this field has been principally due to the development of better analytical techniques. The advent of thin-layer and gas-liquid chromatography in particular has enormously facilitated the purification and identification of complex lipids.

Lipids have a high caloric value and their catabolism yields 2.2 and 1.6 times as much energy per gram as carbohydrates and proteins, respectively. They also yield twice as much metabolic water which plays an important role in terrestrial animals. Much of the food eaten by insects during the immature stages is converted into fat and stored in cells of the fat body.

Blattella germanica L. has long been employed as a subject of physiological and toxicological investigations but lipid biochemistry has been neglected. My purpose was to investigate the qualitative and quantitative changes in the lipid composition of *B. germanica* during embryonic and post embryonic development.

REVIEW OF LITERATURE

Embryonic Stages

During embryonic development important qualitative and quantitative changes may occur in the lipid fraction. In insects, neutral glycerides serve as the major energy source. Needham (1931) proposed a theory of a succession of energy sources during ontogeny; the embryo first utilizing carbohydrate primarily, then protein and finally fat.

A number of studies on lipid content of orthopteran eggs indicate that, in general, the lipid content ranges from 2.5 to 14% of the wet weight. The lipid concentration declines during development of the embryo. A large part of the loss appears to be in the triglyceride fraction; lipids are the major source of energy for the developing embryo. The greatest loss of lipids occurs during the later stages of development.

Probably the earliest work on the lipid content of an orthopteroid egg was that of Dubois (1893), who reported that newly laid eggs of the Algerian locust, *Acridium peregrinum* Oliv. contain 4 to 5% fatty material (wet weight basis) and this was considerably reduced during development. No quantitative estimate of the loss was given. Slifer (1930) reported that 9 to 12% of the wet weight of the newly laid eggs of *Melanoplus differentialis* (Thomas) was fatty acid. During embryogenesis 54% of the initial fatty acid reserve was catabolized. Most of the loss occurred in the post diapause period. The iodine value remained constant during embryonic development. This was confirmed by Boell (1935) and Hill (1945). On the basis of respiratory studies, Boell (1935) calculated the loss of fatty acids as 67% of the initial store. Hill (1945) showed that carbohydrate formed the major energy source during the first 5 days of development. Protein and fat were chiefly used in the prediapause and diapause period. During post diapause fat catabolism accounted for 90% of the oxygen consumed.

Carausius (Dixippus) morosus (Br. and L.) used 26% of the lipid reserve during embryonic development and the fat content was reduced from 31% of the dry matter to 23% at the time of hatching (Lafon 1950).

Blackith and Howden (1961) and Allais et al. (1964) recorded loss of lipids during embryogenesis in *Locusta migratoria* (L.). The latter authors observed that lipids accounted for 26% of the dry weight in the newly laid eggs (78.5% triglycerides, 19.5% phospholipids and 2% sterols) and showed a total decrease of 31.2% to form 20.7% at the end of embryonic development (triglycerides 66%, phospholipids 19.5% and sterols 3%). They concluded that this decline in lipid content was due only to catabolism of triglycerides. Phospholipids consisted chiefly of lecithins (70.5%) and cephalins (26.5%) and a small amount of sphingomyelin. The phospholipid content increased by 60% during embryogenesis, but no qualitative changes were observed. Sterol content remained constant and for the most part was in the free form.

Kinsella and Smyth (1966) and Kinsella (1966a, c, d) made an exhaustive study of the lipids of *Periplaneta americana* L. During embryogenesis, total extractable lipids decreased from 39.5% to 23.2% of the dry weight, mainly due to catabolism of the triglyceride fraction. There was an increase in the mono- and di-glyceride fractions (Kinsella and Smyth 1966).

Sterol content remained constant during development. The sterol esters of newly extruded oothecae contained mainly palmitic, stearic, oleic and linoleic acids. Palmitic and stearic acid content decreased during development (Kinsella 1966d). Sphingomyelin, lecithin and cephalin are the major phospholipids. Small amounts of lysolecithin, phosphatidyl inositol and cerebroside were also found. Total phospholipid content increased fourfold during development. Sphingomyelin and cephalin content tripled and lecithin content doubled (Kinsella 1966a). There was close similarity in the fatty acid composition of the total lipid, neutral lipid and triglyceride fractions. Palmitic, stearic, oleic and linoleic acids content accounted for 95% of the total fatty acids. The phospholipid fraction, however, had a greater amount of linolenic acid (Kinsella 1966c).

The lipid picture for the period of embryogenesis in *Leucophaea maderae* (Fabr.) was characterized by a decreasing content of embryonic triglyceride and an increasing proportion of phospholipids (Gilbert 1967b).

Post Embryonic Stages

The lipids of post embryonic stages of orthopteroid insects vary over a wide range (1.7 to 16% of the wet weight). The immature stages, in general, have a higher lipid content.

Tsujimoto (1929) analyzed the fat of *Oxya japonica* (Fabr.). This species contained 3% fat (dry weight basis) and had a saponification number of 175, an iodine value of 122.6 and 15.7% unsaponifiable matter. Palmitic, stearic, oleic and linoleic acids were identified. Seventy-five per cent of the fatty acids were unsaturated. Body lipids of *Acheta mitrata* constituted 2.4% of the fresh weight. Unsaponifiable matter made up 11.3% of this and contained 45.5% sterols.

Sacharov (1930) reported that 3 to 5 day old nymphs of *L. migratoria* contained 2.8% fat. Matthee (1945) investigated some of the biochemical differences between the solitary and gregarious phases of *L. migratoria* and *Locusta pardalina* Walk. The fat content of *L. migratoria solitaria* adult was 11.0% of the dry weight and increased to 14.0% in *L. migratoria gregaria* . Similarly the fat content of the solitary phase adults of *L. pardalina* increased from 12.8 to 14.6% (dry basis) in the migratory phase. Fawzi, Osman and Schmidt (1961) recorded a much higher fat content in the migratory phase of *L. migratoria*; 10.4% of the wet weight in females and 14.6% of the wet weight in the males.

The lipid content of the German cockroach, *B. germanica* was studied by Mellampy and Maynard (1937). The lipid content of the nymphs, females with egg capsules and males was 5.7, 4.8 and 1.7% of the wet weight respectively. The Iodine Number was 69 for the nymphs and 74 for the females and males. In a later study McCay (1938) reported that of the dry weight of adults 15.6 to 17.1% was ether extractable material.

Lipid content of *P. americana* adults has been studied by a number of investigators. Schweet (1941) reported that the lipid content of adult females and males was 28.6 and 25.5% of the dry weight. According to Munson and Gottlieb (1953) the lipid content of the nymphs, adult males and females was 7.7%, 7.1% and 8.9% of the wet weight respectively. Siakotos and Zoller (1960) and Kinsella and Smyth (1966) reported that

30% of the dry matter was lipids. Lofgren and Cutkomp (1956) recorded much lower values, 13.9% and 14.5% of the dry weight in females and males. Kinsella (1966a, c, d) compared the lipid composition of the nymphs and adults with those of the various stages of the embryo. The lipid composition of nymphs and adults was quite similar. Neutral lipid accounted for 75% of the total lipids. Lecithin, cephalin and sphingomyelin were the predominant phospholipids in that order of abundance (Kinsella 1966a). The fatty acid pattern of the various lipid fractions was similar to those found in the embryo (Kinsella 1966c).

On a wet weight basis, the adults of the grasshopper *Melanoplus atlanis* Riley consisted of 0.8% neutral fat and 2.4% fatty acids (Giral, Giral and Giral 1946). The free fatty acids consisted of stearic, palmitic, arachidic, and unsaturated C_{16}, C_{18}, C_{20} and C_{22} acids. Linolenic acid was not present but triethenoic acids of the C_{20}-C_{22} series appeared to be present. The polyunsaturated acids with more than 18 carbons were present in large amounts (46.2% of the total fat).

Components of the body fat of *Taeniopoda auricornis* Walk. was reported by Giral, Giral and Giral (1943). Fatty acids of the females consisted of 35% saturated acids, 6.5% oleic acid and 58.5% linoleic acid. Fatty acids of the males contained 15.5% saturated acids, 24.0% oleic and 60.5% linoleic acid. The lipids of the females contained 5.1% unsaponifiable matter; of the males 6.5%.

Giral (1946) found that the lipids extracted from the adults of *Sphenarium purpurescens* (Charp.) contained a very high proportion of free fatty acids. Glycerides were present in very small amount. The fatty acids consisted of 22.1% saturated acids, 9.6% palmitoleic acid and 35.5% oleic acid. Of the fatty acids, 25.8% had a chain length of more than 18 carbons.

Barlow (1964) found that palmitic, oleic, linoleic and linolenic acids accounted for 95% of the fatty acids in the body fat of *Melanoplus sanguinipes* (Fab.).

MATERIALS AND METHODS

Laboratory Rearing of the Roaches

The roaches were fed rabbit pellets (3.5 - 4.5% lipids) and were kept in battery jars with pieces of folded paper which served as resting and hiding space. A one pound narrow mouthed jar was used as a water reservoir and a cotton wick was provided, the upper end being wrapped with absorbent cotton to make it fit tightly in the mouth of the bottle. Water and food was replenished once every three weeks. The roaches were raised at a temperature of 30 ± 1 C. Two to three hundred individuals were raised in one jar and adults were collected once every three days. Adults collected on the same day from different jars were pooled, maintained under the same conditions as described above, and used for lipid analysis after one week.

Extraction and Purification of Lipids

The procedure of Folch, Lees and Sloane-Stanley (1957) was followed. The insects were placed in a glass vial with 20 volumes of a chloroform:

methanol mixture (2:1 v/v) and homogenized in a Potter-Elvehjem homogenizer for 30 minutes and filtered through a sintered glass funnel into a glass stoppered vial. The filtrate was shaken for 3 to 4 minutes with 0.2 volumes of 0.90% sodium chloride solution. The mixture was centrifuged for 5 minutes at 400 g and the upper methanol:water:salt layer was removed with a fine pipette. To ensure complete removal of the solutes in the upper phase, the interphase was rinsed three times with a small volume chloroform:methanol:saline (3:47:48 v/v/v). The lower phase was evaporated to dryness in a rotary flash evaporator, dissolved in a small volume of chloroform and stored at -10 C under nitrogen until further use.

Separation of Lipid Class Spectrum

The total lipid extract was placed on a 30 g silicic acid:Hyflo super cell column (2:1 w/w; column dimensions 2.2 x 14 cm) and eluted first with 200 ml of chloroform to obtain the neutral lipids. The phospholipids retained on the column were then eluted with 200 ml of chloroform:methanol (1:1 v/v) mixture. The eluates were then evaporated to dryness and weighed. The neutral lipid fraction was then placed on a 30 g de-activated florisil column (2.2 cm x 15 cm) and the lipid classes were eluted according to the method of Carrol (1961). The solvents of the isolated lipid classes were first removed in a flash point evaporator and were then placed in a vacuum oven at 40 C in tared planchets and the solvents removed. The planchets were reweighed to obtain the weights of the individual classes. The efficiency of the separation of these classes of compounds was checked by thin-layer chromatography (Mangold 1961).

Analysis of Fatty Acids by Gas Liquid Chromatography

Aliquots of lipid fractions were transmethylated by refluxing in 5 ml of 5% sulfuric acid in dry methanol (w/v) for 3 hours (Patton, Durdan and McCarthy 1964). An equal volume of distilled water was added and the methyl esters extracted three times with a small volume of redistilled petroleum ether (30-60C). The combined ether extract was dried over anhydrous sodium sulfate and the petroleum ether removed under a stream of nitrogen. The methyl esters were then dissolved in a small volume of spectranalyzed n-hexane and small aliquots were used for separation by gas-liquid chromatography.

Qualitative and quantitative analyses of fatty acid methyl esters were made with a Beckman Model GC 5 Gas Chromatograph equipped with a dual hydrogen flame ionization detector. Copper columns (15' long, 1/8" O D) containing Chromosorb P (60-80 mesh) coated with DEGS (diethylene glycol succinate, 20% by weight of the solid support) were used. Since the column was continuously maintained at 200 C, the amount of liquid phase in time was reduced. This resulted in reduction of retention time. Standard methyl esters of fatty acids for comparative purposes and quantitation were obtained from Mann Research Laboratories. Unknown peaks were tentatively identified by logarithmic plot by the method of James (1959).

Thin Layer Chromatography of Phospholipids

Phospholipid content was determined by silicic acid Hyflo super cell chromatography. The individual phospholipid classes were seperated by thin-layer chromatography on 0.5 mm thick Silica gel G plates according to Wagner, Horhammer and Wolff (1961). The developing solvent was chloroform:methanol:water (65:25:4 v/v). The developing chamber was lined with filter paper saturated with the solvent mixture. Following the chromatographic run, the thin-layer plates were air dried and exposed to iodine vapour for visualization of the phospholipids. Identification of the individual phospholipid was accomplished by comparison of the R_f with those of pure standards obtained from Applied Science Laboratories, State College, U.S.A. and specific color reactions. Quantitation of the major phospholipids was based on the phosphorus content, determined colorimetrically according to the method of Bartlett (1959). Three determinations were made for each stage.

RESULTS

Dry Matter Content

The wet weight, dry weight (obtained by drying at 100 C) and water content of the ootheca, nymphs and adults of the German cockroach are given in table 1. The proportion of water in the egg was initially about 62%. This remained constant during the first five days of embryonic development and increased to about 75% by the 15th day. When calculated on a per egg basis, the wet weight of the egg increased from 1.276 mg to 1.617 mg, while the dry matter decreased from 0.486 mg to 0.399 mg (table 2). There was a slight drop in the per cent water content between the 1st and 3rd nymphal instars. In the subsequent nymphal instars and the adult water content remained more or less constant.

Total Lipids

Table 2 reveals that the total lipid content of the eggs decreased during embryonic development. The newly formed eggs, on an average, contained 0.178 mg of lipids which in 15 days of embryonic development decreased to 0.112 mg, representing a loss of 37% of the original lipid component. During the first 5 days of embryonic development, lipid catabolism accounted for 50% of the loss of dry matter; between the 5th and 10th day 56% of the loss of dry matter was due to lipid loss; nearly all of the dry matter loss between the 10th and 15th day of incubation was due to lipid utilization. This indicates that during the early part of embryonic development, some other component(s) must have been used for fulfilling the energy requirements.

There was a progressive increase in the amount of lipids per individual insect as the development of the nymphs proceeded, reaching a maximum in the 6th instar (table 2). However, the lipid content dropped considerably in the adult stage. The females had twice as much lipid as the males.

TABLE 1. Wet weight, dry weight and water content at the various stages of embryonic and post embryonic development of *B. germanica*, means ± S. D. based upon 10 replicates.

Stage of development	Wet wt. (mg)	Dry wt. (mg)	Water content (%)
Ootheca			
0 days	38.29 ± 0.29	14.58 ± 0.25	61.93 ± 0.38
5 days	37.55 ± 0.26	14.29 ± 0.29	61.94 ± 0.31
10 days	43.25 ± 0.27	13.60 ± 0.17	68.55 ± 0.47
15 days	48.50 ± 0.16	11.98 ± 0.27	75.30 ± 1.35
Nymphs			
1st instar	2.25 ± 0.10	0.62 ± 0.07	72.58 ± 0.85
2nd instar	5.09 ± 0.14	1.49 ± 0.07	71.31 ± 1.86
3rd instar	9.92 ± 0.16	2.99 ± 0.09	69.86 ± 0.89
4th instar	20.47 ± 1.42	6.51 ± 0.37	68.19 ± 1.56
5th instar	36.85 ± 1.38	11.59 ± 0.29	68.52 ± 1.21
6th instar	53.48 ± 2.45	16.93 ± 0.94	68.54 ± 1.52
Adults			
♂	47.72 ± 1.14	15.54 ± 0.36	67.41 ± 1.13
♀	64.78 ± 0.98	19.79 ± 1.51	69.44 ± 2.46

Lipid Class Spectrum

At the beginning of embryonic development the neutral lipid constituted 94.6% of the total lipid, but by the time the eggs were 15 days old, it decreased to 86% of the total lipid content (table 2). Embryonic development resulted in a 43% loss of the neutral lipid reserve. During nymphal growth the neutral lipid content per individual increased more or less proportionally to the increase in body weight, reaching a maximum in the 6th instar. The adults have a lower neutral lipid content than the last instar nymphs.

The neutral lipid was fractionated into the following classes: hydrocarbons, free sterols, sterol esters, triglycerides, diglycerides, monoglycerides and free fatty acids. Table 3 is a summary of the analyses conducted at the various stages of development. The values are based on three analyses for each stage. This table shows that the neutral lipid fraction consisted predominantly of triglycerides in all the stages. During embryonic development the level of triglyceride fell from 94% to 76% of the neutral lipid fraction. Gravimetrically there was a 54% loss of the triglyceride content. The loss in the triglyceride content (2.58 mg) was greater than the loss of dry matter (2.51 mg) and neutral lipid (2.16 mg). The proportion of mono- and di-glyceride as well as the free fatty acid content increased considerably during incubation. Quantitatively (table 4) the mono- and di-glyceride content increased 6 and 10 times of the

TABLE 2. Wet and dry weight, total lipid, phospholipid and neutral lipid content per individual during embryonic and post embryonic development of *B. germanica* L.

				Lipid content**			Phospholipid**		Neutral lipid**	
Stage of development	Wet wt* (mg)	Dry wt* (mg)	Water content (%)*	mg	% of wet wt	% of dry wt	mg	Total lipids (%)	mg	Total lipids (%)
Egg										
newly formed	1.276	0.486	61.9	0.178	14.0	36.6	0.010	5.4	0.168	94.6
5 day old	1.252	0.476	61.9	0.173	13.9	36.6	0.011	6.3	0.162	93.7
10 day old	1.442	0.453	68.5	0.160	11.1	35.3	0.014	8.6	0.146	91.4
15 day old	1.617	0.399	75.3	0.112	6.9	28.1	0.016	13.9	0.096	86.1
Nymph										
1st instar	2.25	0.62	72.6	0.123	5.5	19.8	0.021	17.3	0.102	82.7
2nd instar	5.09	1.49	71.3	0.279	5.5	18.7	0.054	19.7	0.225	80.3
3rd instar	9.92	2.99	69.9	0.570	5.8	19.1	0.095	16.7	0.475	83.3
4th instar	20.47	6.51	68.2	1.402	6.9	21.5	0.230	16.4	1.172	83.6
5th instar	36.85	11.59	68.5	2.141	5.8	18.5	0.363	17.4	1.768	82.6
6th instar	53.48	16.93	68.5	3.920	7.3	23.2	0.758	19.3	3.162	80.7
Adult										
♂	47.72	14.54	67.4	1.933	4.1	12.4	0.343	17.8	1.590	82.2
♀	64.78	19.79	69.4	3.699	5.7	28.9	0.658	17.8	3.041	82.2

* *Values presented are means of 10 replicates. These data appear also in table 1.*

** *Values presented are means of 3 replicates.*

initial concentration per individual respectively. The free fatty acid content increased 7 fold.

TABLE 3. Proportion of various lipid classes in the neutral lipid fraction of the German cockroach (% of neutral lipid fraction), means of three replicates for each stage.

Stage	Hydro- carbons	Sterol esters	Tri- glycerides	Sterols	Di- glyceride	Mono- glyceride	Free fatty acid
Ootheca							
0 day	1.16	0.89	94.35	2.49	0.24	0.55	0.32
5 day	1.27	1.05	91.84	2.46	1.00	1.52	0.86
10 day	1.62	1.44	88.51	2.17	1.83	2.31	2.12
15 day	2.56	3.21	75.87	3.49	4.29	5.63	4.94
Nymphs							
1st instar	5.90	6.06	70.12	5.33	4.53	4.17	3.89
2nd instar	5.45	5.33	71.21	5.59	4.64	4.04	3.74
3rd instar	5.26	5.64	70.09	5.93	5.63	4.52	2.93
4th instar	4.97	6.03	69.40	5.84	5.71	4.95	3.10
5th instar	7.85	5.78	69.77	5.07	2.83	4.64	4.06
6th instar	5.18	4.40	69.88	7.39	4.52	4.79	3.84
Adults							
♂	6.35	5.70	66.71	7.02	4.97	4.88	4.36
♀	6.40	6.41	71.94	4.48	4.42	3.90	2.45

The relative proportion of the various fractions remained more or less constant in all the nymphal stages. Quantitatively, all the fractions increased in each successive nymphal instar (table 4). Adult females had a neutral lipid class spectrum similar to the nymphs. Adult males had a slightly lower triglyceride level compared to other post embryonic stages.

Sterol content was the same on day 5 as on day 0 of embryonic development. There was a drop between the 5th and 10th day but an increase to a higher amount by the 15th day (table 4). The proportion of esterified sterol progressively increased from 26% in the newly formed oothecae to 48% in the 15 day old oothecae (table 3). Total sterol content increased in successive nymphal instars. The proportion of free and esterified sterol fluctuated. Sterol of females was predominantly in the esterified form while in the males the free forms predominated.

Hydrocarbon content increased gradually at successive developmental stages of the embryo. During nymphal development, the increase in the hydrocarbon content more or less paralleled the increase in the total

TABLE 4. Lipid class spectrum of the neutral lipid fraction during various stages of *B. germanica*, means of three replicates. Weights in mg per ootheca, nymph, and adult.

	Hydro-carbons	Sterol esters	Sterols (free)	Total sterols	Tri-glycerides	Di-glycerides	Mono-glycerides	Total glycerides	Free fatty acids
Ootheca									
0 days	.059	.045	.126	.171	4.775	.012	.028	4.815	.016
5 days	.062	.051	.120	.171	4.481	.049	.074	4.604	.042
10 days	.071	.063	.095	.158	3.876	.080	.010	4.057	.093
15 days	.074	.093	.101	.194	2.195	.124	.163	2.482	.143
Nymphs									
1st instar	.006	.006	.005	.011	0.072	.005	.004	0.081	.004
2nd instar	.013	.012	.013	.025	0.160	.010	.009	0.179	.008
3rd instar	.025	.027	.028	.055	0.333	.027	.021	0.381	.014
4th instar	.058	.071	.068	.139	0.814	.067	.058	0.939	.036
5th instar	.139	.102	.090	.192	1.233	.050	.082	1.365	.072
6th instar	.164	.139	.234	.373	2.210	.143	.151	2.504	.121
Adults									
♂	.100	.091	.112	.203	1.061	.079	.078	1.218	.069
♀	.195	.195	.136	.331	2.188	.134	.118	2.440	.075

lipid content.

Phospholipids

The total phospholipid present in each stage is shown in table 2. The per cent phosphorus content of the major phospholipid fractions (lecithins, cephalins and sphingomyelin) at various stages of development are recorded in table 5. From this the amount of the major phospholipid fractions (in terms of phosphorus content) were calculated and shown in table 5.

During the embryonic development total phospholipid content as well as the three major fractions increased. The proportion of phospholipid at the beginning of embryonic development averaged 6% of the total lipid content. In the 15 days of embryonic development it rose to 13% of the total lipid. Gravimetrically the total phospholipid content increased one and a half times. This indicates a synthesis of phospholipids. Once the nymphs had started feeding, the increment in the phospholipid content was in proportion to the increase in total fat content. During the six subsequent nymphal instars the level of phospholipids remained nearly constant at about 18% of the total lipids.

Phosphatidyl choline (lecithin) and phosphatidyl ethanolamine (cephalin) were the principal phospholipids. In addition to the above two and sphingomyelin, which were quantitated, phosphatidyl serine, phosphatidyl inositol, lysolecithin and phosphatidic acid were detected in trace amounts in all stages studied. During embryonic development the lecithin/cephalin ratio decreased. However, the total increase in lecithin content was greater than the increase in cephalin. The relative proportion of the three major phospholipid fractions remained constant in the nymphal stages. Based on lipid phosphorus, the per cent composition of lecithin, cephalin and sphingomyelin was 57, 33 and 6% respectively. The lecithin/cephalin ratio varied from 1.63 to 1.77. The phospholipid composition of males and females was similar.

Fatty Acid Composition

Figure 1 shows an analysis of fatty acids from an adult female sample. This analysis revealed the presence of 17 fatty acids ranging in carbon chain length from 6 to 22. Of these $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$ mixed with $C_{20:0}$ were quantitated. With the column used $C_{18:3}$ and $C_{20:0}$ could not be separated. Table 6 represents a summary of the gas chromatographic analysis of the fatty acids in the total lipid extract at 12 different stages of development.

Oleic acid ($C_{18:1}$) was the largest component in all the stages and accounted for 42 to 48% of the total fatty acid fraction (table 6). Palmitic acid ($C_{16:0}$) was next in order of abundance. About 70% of the fatty acids were unsaturated. Besides oleic acid, linoleic acid ($C_{18:2}$) was present in fair amount (16 to 19%). Of the remaining 30% saturated fatty acids, palmitic acid ($C_{16:0}$) was the most abundant (25 to 30%).

The proportion of myristic acid ($C_{14:0}$) in the nymphs and adults was three times as much as in the embryonic stages. Palmitic acid content in the 0 and 5 day old oothecae was quite similar but it dropped to a lower percentage in the 10 and 15 day old oothecae. The reverse

TABLE 5. Changes in the distribution of lipid phosphorus and phospholipid fractions during embryonic and post embryonic development of *B. germanica*, means of 3 replicates.

Stage	Lipid phosphorus content		Distribution of phosphorus in major fractions							Lecithin/ cephalin ratio
			% distribution				µg/insect			
	%	µg/insect	Sphingo-myelin	Lecithin	Cephalin	Misc.	Sphingo-myelin	Lecithin	Cephalin	
Egg										
0 day	3.68	0.368	5.5	60.0	31.7	2.8	0.020	0.221	0.117	1.89
5 day	3.75	0.413	5.6	59.1	33.7	1.6	0.023	0.244	0.139	1.75
10 day	3.74	0.524	5.8	58.6	34.2	1.4	0.030	0.307	0.179	1.71
15 day	3.88	0.621	5.7	57.4	34.3	2.6	0.035	0.356	0.213	1.67
Nymph										
1st instar	3.97	0.834	5.7	56.4	34.7	3.2	0.048	0.470	0.289	1.63
2nd instar	3.90	2.106	5.8	56.4	33.7	4.1	0.122	1.188	0.710	1.67
3rd instar	3.89	3.696	6.2	56.6	32.9	4.3	0.229	2.092	1.216	1.72
4th instar	4.00	9.200	5.5	58.0	32.7	3.8	0.506	5.336	3.008	1.77
5th instar	3.78	13.731	5.7	55.8	33.8	4.7	0.782	7.656	4.638	1.65
6th instar	4.02	30.472	5.7	56.9	33.4	4.0	1.737	17.339	10.178	1.70
Adult										
♂	3.98	13.651	5.7	56.6	33.9	3.8	0.773	7.676	4.628	1.67
♀	4.00	26.320	5.5	56.5	33.9	4.1	1.448	14.871	8.922	1.67

was the case with oleic acid. The percentage of the remaining major fatty acids were quite similar in all the fatty acid composition between sexes. During nymphal growth, the proportion of palmitic and palmitoleic acid increased, but the proportion of linoleic and linolenic acid decreased.

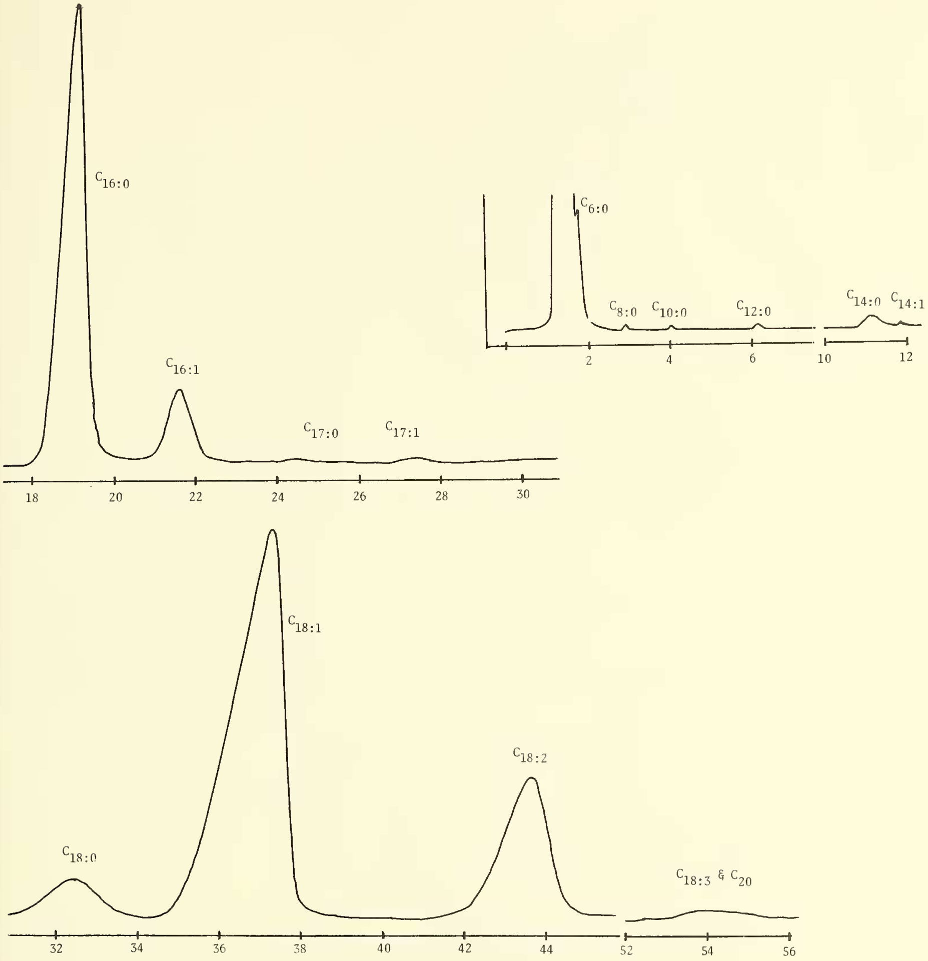

Fig. 1. Gas chromatographic separation and identification of fatty acid methyl esters of total lipids of adult female *B. germanica*. $C_{6:0}$ caproate; $C_{8:0}$ caprylate; $C_{10:0}$ caprate; $C_{12:0}$ laurate; $C_{14:0}$ myristate; $C_{14:1}$ myristoleate; $C_{16:0}$ palmitate; $C_{16:1}$ palmitoleate; $C_{17:0}$ heptadecanoate; $C_{17:1}$ heptadecenoate; $C_{18:0}$ stearate; $C_{18:1}$ oleate; $C_{18:2}$ linoleate; $C_{18:3}$ linolenate; $C_{20:0}$ arachidate. Instrument Settings: Column, 15' x 1/8" OD, 20% diethylene glycol succinate on Chromosorb P; Detector, hydrogen flame ionization; Detector temperature, 240 C; Injection port temperature, 250 C; Column temperature, 200 C; Hydrogen flow rate, 25 ml per minute; Carrier gas and flow rate, helium 40 ml per minute.

TABLE 6. Fatty acid composition of the total lipid extract of *B. germanica* L. at various stages of the life cycle.

Stage	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$ & $C_{20:0}$
Ootheca							
Newly formed	0.4 ± 0.1*	29.1 ± 0.9	4.2 ± 0.3	2.3 ± 0.3	44.2 ± 1.9	17.6 ± 2.7	2.2 ± 0.3
5 day old	0.3 ± 0.1	30.7 ± 0.8	4.6 ± 0.3	2.1 ± 0.1	45.5 ± 0.3	15.3 ± 0.4	1.5 ± 0.3
10 day old	0.3 ± 0.1	25.6 ± 0.9	3.6 ± 0.2	3.6 ± 1.1	48.3 ± 0.1	16.5 ± 0.3	2.1 ± 0.9
15 day old	0.4 ± 0.1	25.1 ± 1.3	4.2 ± 0.2	3.3 ± 0.2	47.4 ± 0.7	17.7 ± 0.2	2.0 ± 0.6
Nymph							
1st instar	0.9 ± 0.1	24.2 ± 0.7	3.4 ± 0.4	4.7 ± 0.6	45.5 ± 1.2	19.2 ± 3.1	2.1 ± 0.7
2nd instar	0.9 ± 0.1	26.1 ± 2.1	3.8 ± 1.0	4.4 ± 0.5	44.7 ± 2.2	17.6 ± 0.5	2.5 ± 0.6
3rd instar	1.0 ± 0.0	28.8 ± 0.4	4.9 ± 0.2	3.6 ± 0.1	42.2 ± 0.4	17.0 ± 0.5	2.5 ± 0.6
4th instar	1.0 ± 0.1	28.2 ± 0.6	5.2 ± 0.2	3.7 ± 0.7	43.5 ± 2.5	16.5 ± 1.9	1.9 ± 0.5
5th instar	1.2 ± 0.2	29.4 ± 1.3	6.1 ± 0.6	3.4 ± 0.4	42.2 ± 1.8	15.7 ± 0.7	1.9 ± 0.1
6th instar	1.1 ± 0.1	27.9 ± 2.5	4.8 ± 1.8	3.6 ± 0.8	43.8 ± 1.1	16.7 ± 2.4	2.1 ± 0.3
Adult							
♂	0.9 ± 0.1	24.7 ± 1.0	4.1 ± 0.3	3.5 ± 0.4	45.9 ± 1.6	18.2 ± 1.9	2.7 ± 1.1
♀	0.8 ± 0.1	27.1 ± 1.2	4.6 ± 0.3	3.3 ± 0.1	45.4 ± 1.2	17.0 ± 1.1	1.8 ± 0.3

* *Mean of three samples ± standard deviation.*

$C_{14:0}$ myristic acid; $C_{16:0}$ palmitic acid; $C_{16:1}$ palmitoleic acid; $C_{18:0}$ stearic acid; $C_{18:1}$ oleic acid; $C_{18:2}$ linoleic acid; $C_{18:3}$linolenic acid; $C_{20:0}$ arachidic acid.

Fatty acids contained in the isolated neutral and phospholipid fractions of the 4th instar nymphs, adult males and females were also separated by gas-liquid chromatography. Qualitatively there was no difference between the three types of lipids. Only 8 fatty acids were quantitated. Table 7 shows the relative proportion of these fatty acids. A comparison of the data shows that the proportion of these fatty acids in the neutral lipid fraction approximated the composition in the total lipid extract in all the 3 stages studied. Fatty acid composition of phospholipid fraction showed some differences. The proportion of myristic, palmitic and palmitoleic acids was reduced to half the relative proportion of these acids in total and neutral lipid fractions. The proportion of linoleic acid, on the other hand, was doubled. The phospholipid also contained slightly higher proportion of linolenic and arachidic acids. The fatty acids of the neutral lipid fraction had a higher proportion of saturated fatty acids (40%) than phospholipids (28%).

TABLE 7. Per cent fatty acid composition of the total lipid, and isolated neutral and phospholipid fractions of *B. germanica*.*

	Stage								
	4th instar nymph			Adult males			Adult females		
Fatty acid	TL	NL	PL	TL	NL	PL	TL	NL	PL
$C_{14:0}$	1.0	1.3	0.6	0.9	0.7	0.5	0.8	1.0	0.4
$C_{16:0}$	28.2	30.6	14.5	24.7	28.6	18.3	27.1	31.8	15.0
$C_{16:1}$	5.2	5.6	3.1	4.1	3.8	3.7	4.6	4.5	2.9
$C_{18:0}$	3.7	3.7	6.1	3.5	3.4	3.7	3.3	2.7	4.2
$C_{18:1}$	43.5	41.4	43.3	45.9	45.0	44.9	45.4	44.0	45.9
$C_{18:2}$	16.5	15.3	30.2	18.2	16.4	25.8	17.0	14.3	27.3
$C_{18:3}$ & $C20:0$	1.9	2.1	2.2	2.7	2.1	3.1	1.8	1.7	4.3

* *Values presented are the means of 3 replicates. TL - total lipid; NL - neutral lipid; PL - phospholipid.*

C14:0 myristic acid; $C_{16:0}$ *palmitic acid;* $C_{16:1}$ *palmitoleic acid;* $C_{18:0}$ *stearic acid;* $C_{18:1}$ *oleic acid;* $C_{18:2}$ *linoleic acid;* $C_{18:3}$ *linolenic acid;* $C_{20:0}$ *arachidic acid.*

DISCUSSION

Data presented indicate a progressive increase in the wet weight and a decrease in the dry matter content during embryogenesis. The changes reported here agree, in general, with those of Roth and Willis (1955a, b). Ross (1929) and Parker and Campbell (1940) suggested that the wall of the ootheca of *B. germanica* in contact with the female's genital pouch may be permeable to water. Roth and Willis (1955b) demonstrated a difference in permeability between the anterior and posterior end of the ootheca of *B. germanica*. The anterior end, held by the female is lighter in color and less sclerotized, was more permeable. They concluded that the increase in wet weight was due to absorption of water from the female. A similar phenomenon has been observed in *Blattella vaga* Hebard (Roth and Willis 1955b) and *Diploptera dytiscoides* (Serv.) (Roth and Willis 1955c).

The loss of dry matter was accompanied by a loss in the total lipid content. On the average, 75% of the loss of dry matter was accounted for by lipid catabolism. Many workers who studied the embryonic period have found that fat forms the main source of energy of the developing embryo (Tichimirov 1885, Rudolfs 1926, Fink 1925, Slifer 1930, Busnell 1937, Lafon 1959, Rainey 1950, Rothstein 1952, Gilbert and Schneiderman 1961, Kinsella and Smyth 1966, Gilbert 1967b). The only exception was *Tenebrio molitor* L. which utilized glycogen for energy requirements (Ludwig and Ramazzotto 1965). The loss in the initial lipid supply was comparable to the general average of 55% reported by Needham (1931) for various terrestrial animals.

A comparison of the neutral lipid content of newly extruded ootheca and 15 day old ootheca shows a 43% reduction of the initial supply. Of the various lipid classes of the neutral lipids, only triglyceride shows considerable reduction. There is an increase in the mono- and di-glyceride content. These findings agree with those of Slifer (1930), who showed that 54% of the neutral lipids are catabolized by *M. differentialis* during embryonic development. Tichimirov (1885) observed that neutral glyceride fatty acids diminished by 46% during embryonic development in *Bombyx mori*. *Malacosoma americana* (Fab.) utilized 87% of neutral lipids during incubation (Rudolfs 1926). Gilbert and Schneiderman (1961) reported that the moth *Hyalophora cecropia* (L.) catabolized 57% of the initial neutral lipids during incubation. The Japanese beetle, *Popillia japonica* Newman loses 58% of the initial neutral lipid content during incubation. Allais et al. (1964) showed 53% loss of neutral lipid moiety. Kinsella and Smyth (1966) and Gilbert (1967b) observed 55% loss of neutral lipids during embryogenesis in *P. americana* and *L. maderae*.

In the present work it has been shown that the hydrocarbon and sterol content increased only slightly. With the development of the embryo, the proportion of the esterified sterols increases. Tichimirov (1885), Allais et al. (1964) and Gilbert (1967b) reported similar findings during embryonic development of *Bombyx mori* (L.), *L. migratoria* and *L. maderae* respectively, whereas Rudolfs (1926) reported that the cholesterol in the eggs of *M. americana* disappeared by the time of hatching. Kinsella (1966d) and Gilbert (1967b) also observed an increase in the proportion of esterified sterols during incubation. The more or less constant sterol content ties well with the theory that insects lack the ability to synthesize sterols (Black et al. 1956). However, it is rather difficult to correlate it with the known function of sterols, being a major constituent of cellular membranes. During morphogenesis, it would be expected to increase, unless the sterol stored in the yolk is sufficient to meet the requirements for membrane formation.

During nymphal growth and in the adults the sterol content increases in proportion to the increase of the total fat content. It has been shown that some dietary sterols can be converted into cholesterol(Gilmour 1961).

During nymphal development, all the lipid fractions increase. In order to compare the rate of increase of lipids with that of wet weight, log of wet weight and log of total lipids of the different nymphal instars were plotted and a straight line was drawn. The total amount of fat was related to the wet weight by the equation $y = b x^k$, where k is known as the heterauxetic constant. The same formula may also be written in the logarithmic form $\log = \log b + k \log x$. The value of k was close to 1 indicating that the rate of accumulation of fat during nymphal growth occurs more or less at the same rate as that of the total body weight. Similarly, k values were calculated for neutral lipids against total lipids, phospholipids against total lipids, triglycerides against neutral lipids, sterols with neutral lipids and hydrocarbons with neutral lipids (fig. 2a-h). In all the cases the k value was close to unity indicating that all the rate of increase of each fraction paralleled the increase in the other fraction. In holometabolous insects, on the other hand, the k value is greater than unity (Finkel 1948, Fast 1964) indicating that the weight of lipid increases more rapidly during larval growth than the total weight. Accumulation of large amount of fat is advantageous for holometabolous insects, because considerable energy is required for transformation into adult. In addition many adults do not feed and depend upon fat reserves.

The fatty acid composition of *B. germanica* closely resembles that of *P. americana* (Kinsella 1966c). The predominance of oleic acid and palmitic acid is in conformity with other insects (Fast 1964). Only aphids and coccids are peculiar in having a large proportion of myristic acid and low level of oleic acid (Strong 1963, Barlow 1964, Fast 1964). Only trace amounts of fatty acids having carbon chain length more than 20 were found in *B. germanica* . This is in agreement with the observations of most workers. Giral (1946) and Giral, Giral and Giral (1946) reported 25.8% and 46.2% of fatty acids were more than 20 carbon chains long in *S. purpurescens* and *M. atlanis* respectively. Albrecht (1961) reported 72% stearic acid in *Schistocerca gregaria* Forsk. which is a very high value when compared with the iodine number. Usually stearic acid content in insects is lower than 10% (Fast 1964).

The fatty acid composition of all the nymphal instars and the adults are quite similar. These results may be interpreted to mean that there is no selective synthesis or accumulation of any fatty acids during nymphal growth.

The loss in triglyceride is greater than dry matter loss during embryonic development. It is possible that part of the triglycerides are hydrolyzed to 1,2 diglyceride and utilized for the synthesis of phospho-

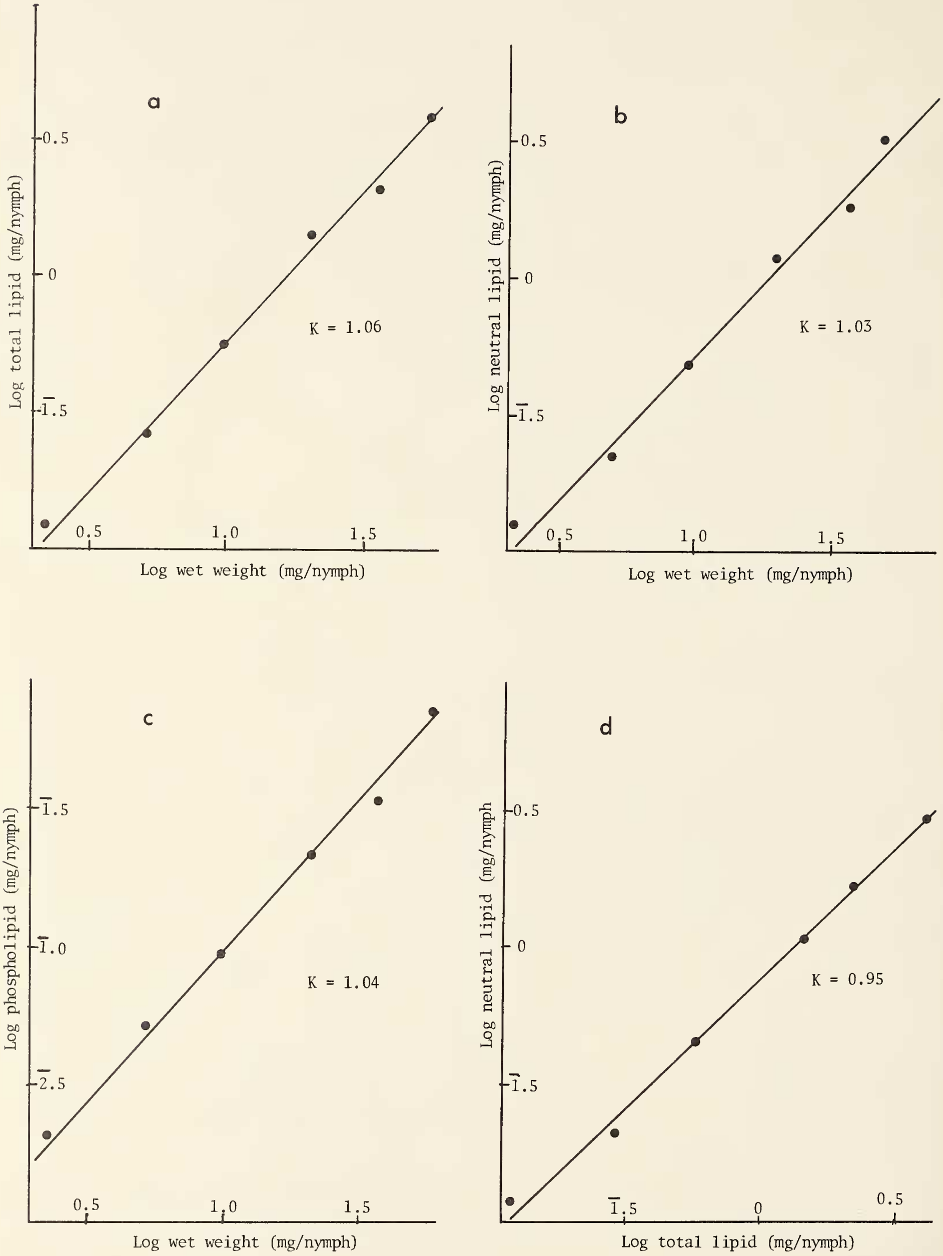

Fig. 2 (a-d). Double-log plot of (a) total lipid against wet weight; (b) neutral lipid against wet weight; (c) phospholipid against wet weight and (d) neutral lipid against total lipid. Graphs are from data in table 2.

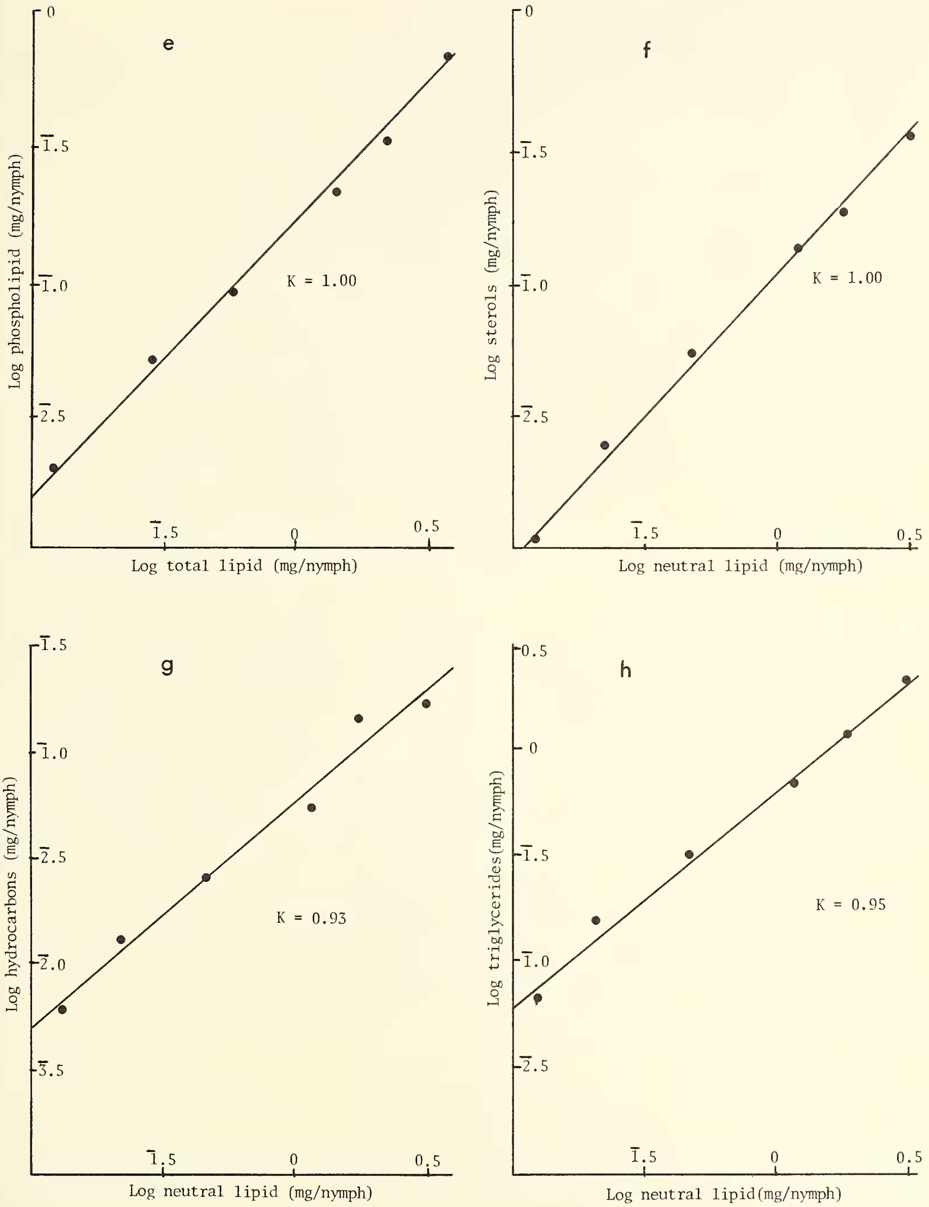

Fig. 2 (e-h). Double-log plot of (e) phospholipid against total lipid; (f) sterols against neutral lipid; (g) hydrocarbons against neutral lipid and (h) triglycerides against neutral lipid. Graphs are from data in table 2 and 4.

lipids via phosphatidic acid. Part of the loss is accounted for by the increase in mono- and di-glyceride content during embryonic development (Kinsella and Smyth 1966, Gilbert 1967b). The fatty acids released from the glycerides may be oxidized completely or utilized for the synthesis of sterol esters.

The phospholipid content increases during embryonic and post embryonic development. During incubation, the increase in the proportion of phospholipids has been shown to be partially due to synthesis and partially due to utilization of triglycerides for energy requirements. Phospholipids are parts of cellular and subcellular membranes (Ansell 1964) and hence will increase during morphogenesis. Similar lipid patterns have been reported by Tichimirov (1885) in *M. americana*; by Pearincott (1960) in *Musca domestica* L.; Bieber et al. (1961) in *Phormia regina* (Meigen); Allais et al. (1964) in *L. migratoria*; Kinsella (1966a) in *P. americana*; and Gilbert (1957b) in *L. maderae*.

The relative proportion of the three major phospholipid classes lecithin, cephalin and sphingomyelin was quite similar to that reported by Allais et al. (1964) in *L. migratoria* and Siakotos and Zoller (1960) and Kinsella (1966a) in *P. americana*. Many dipterans are characterized by the predominance of cephalin over lecithins (Fast 1964).

When calculated on a per individual basis, the lipid phosphorus content increased throughout development as a result of incorporation of non-lipid phosphorus into the phospholipid fraction. Chojnacki (1961) and Chojnacki and Piechowska (1961) studied the mechanism of synthesis in *Celerio euphorbiae* (Fab.) and found it to be similar to biosynthesis in vertebrate liver. Phosphocholine (or phosphoethanolamine) is activated by reaction with cytidenetriphosphate to yield cytidinediphosphate-choline (or ethanol-amine) intermediate which then reacts with a, b diglyceride to yield choline (or ethanolamine) phosphatide.

The three roach species whose lipid metabolism during embryogenesis has been studied, have different oviposition habits. In *P. americana* the ootheca is extruded and carried by the female for only a short period and then deposited. In *B. germanica* the ootheca is extruded and carried by the female until the eggs hatch. In *L. maderae* the ootheca is extruded, then subsequently retracted into a brood sac until or shortly before hatching (Roth and Willis 1954). The incubation period of *P. americana* is twice as long as that of the other two species. The lipid metabolism pattern is, however, similar in all the three species. Thus oviposition or incubation period has very little effect on lipid metabolism.

SUMMARY

During the embryonic development of *B. germanica* there is an increase in the moisture content and a decrease in the dry matter and lipid content. The reduction in the lipid content was due to catabolism of triglycerides. The neutral lipid and triglyceride content decreased by 43% and 54% respectively. The decrease occurs mostly in the second half of development. On the other hand, the mono- and di-glyceride content increased throughout incubation. Phospholipid content increased by 60% during embryo-

genesis. The major components of the phospholipid fraction are phosphatidyl choline, phosphatidyl ethanolamine and sphingomyelin. In addition phosphatidyl inositol, lysolecithin and phosphatidic acid were present in small amounts. Hydrocarbon and sterol content showed slight increase. With the progress of embryonic development the proportion of esterified sterols increased. The overall decrease of 75% of the initial lipid store shows that lipids play a dominant role in fulfilling the energy requirements of developing eggs.

During nymphal development, the insect accumulates large amounts of lipid. Increase in the total lipid, neutral lipid and phospholipid content is proportional to the increase in the wet weight of the body. Though the adults had a lower fat content than the last instar nymphs, the lipid patterns are similar. In all the stages studied, triglycerides were the predominant fraction.

Fatty acid analysis of the phospholipid, neutral lipid and total lipid extracts revealed the presence of 17 fatty acids during all stages of the life cycle ranging in carbon chain length from 6 to 22. Palmitic, oleic and linoleic acids were the major fatty acids. Unsaturated fatty acids predominated in the various fractions with oleic acid comprising about 45% of the total fatty acids in all the fractions studied. Palmitic acid was the second most abundant in the neutral lipid fraction, but linolenic was the second most abundant in the phospholipid fraction. Fatty acids of the phospholipids were more unsaturated than the fatty acids of the neutral lipids.

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